

REPORT

DETERMINATION OF THE WATER SOLUBILITY OF



**NOTOX Project 338603
NOTOX Substance 111834/C**

STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with the most recent edition of:

The OECD Principles of Good Laboratory Practice

which are essentially in conformity with:

The United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

The United States Environmental Protection Agency (FIFRA). Title 40 Code of Federal Regulations Part 160.

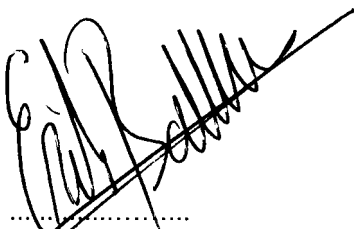
The United States Environmental Protection Agency (TSCA). Title 40 Code of Federal Regulations Part 792.

CONFIDENTIALITY STATEMENT

This report contains the unpublished results of research sponsored by [REDACTED]
[REDACTED] Reproduction, issue or disclosure to third parties in any form is not permitted without prior written authorisation from the sponsor.

Study Director

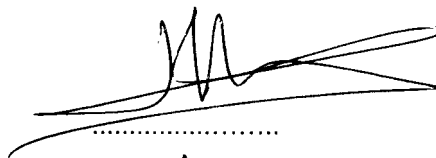
Dr.Ir. E. Baltussen



Date: 18 JUL 2002

Management

Dr. Ir. H. Willems
Section Head
Analytical & Physical Chemistry



Date: July 18, 2002

QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was audited by the NOTOX Quality Assurance Unit to ensure that the methods and results accurately reflect the raw data.

The dates of Quality Assurance inspections and audits are given below.
During the on-site inspections procedures applicable to this type of study were inspected.

DATES OF QAU INSPECTIONS/AUDITS**REPORTING DATES****on-site inspection (s)**

13 May to 31 May 2002 (Process, Physical Chemistry) 04 June 2002

protocol inspection (s)

06 November 2001 (Study)

06 November 2001

report audit (s)

18 June 2002 (Study)

18 June 2002

Head of Quality Assurance
C.J. Mitchell B.Sc.



Date: 19-7-02

SUMMARY

Guidelines and test method

The flask method was used to determine the water solubility of Trigonox R-938 and was based on the following guidelines:

OECD guideline for testing of chemicals no. 105: "Water Solubility", Adopted July 27, 1995.

EEC Directive 92/69 EEC, Part A, Methods for the determination of physico-chemical properties, A.6: "Water solubility". EEC Publication no. L383, December 1992.

Quantification

According to information supplied by the sponsor, [REDACTED] is a formulation containing several components (see the certificate of analysis). Measurements were performed on the following components: [REDACTED] Due to the fact that DMP, which was used for preparation of this formulation, is a very well known compound, which cannot be determined in the same chromatographic run as the other compounds due to a large difference in sensitivity (see also Notox Project 338805 "Development and validation of an analytical method for [REDACTED]"), analyses were not based on this compound.

Test results

After each stirring period (17 days; preliminary test or 24, 48 or 72 hours; main study), there was still a two-phase system (water and test substance). Test temperature: $20.0 \pm 0.5^{\circ}\text{C}$ (preliminary test) and $20.0 \pm 0.4^{\circ}\text{C}$ (main study). Analytical measurements on each of the components of concern in the water phase gave the following results:

During the test, hydrogen peroxide is formed as a result of the reaction of the other peroxidic compounds with water. Based on Hydrogen peroxide, [REDACTED] was determined to be miscible with water in at least a 1:1 (v:v) ratio.

During the test MIPK is formed as a result of the reaction of the other peroxidic compounds with water. Based on MIPK, the water solubility of [REDACTED] was determined to be ≥ 230 g/l.

Based on MIPKP-T4, the water solubility of [REDACTED] was estimated to be ≥ 201 g/l.

Based on MIPKP-T3 peak 1, the water solubility of [REDACTED] was determined to be 2.53 g/l.

Based on MIPKP-T3 peak 2, the water solubility of [REDACTED] was determined to be 1.73 g/l.

PREFACE

Sponsor	[REDACTED] [REDACTED] [REDACTED] [REDACTED]
Study Monitor	[REDACTED] [REDACTED]
Testing Facility	NOTOX B.V. Hambakenwetering 7 5231 DD 's-Hertogenbosch The Netherlands
Study Director	Dr. Ir. E. Baltussen
Study plan	Start: 23 April 2002 Completed: 31 May 2002

TEST SUBSTANCE

Identification	[REDACTED]
Chemical name	[REDACTED]
CAS RN	[REDACTED] [REDACTED]
Description	Clear colourless liquid
Batch	[REDACTED]
Purity	See Certificate of Analysis
Test substance storage	In refrigerator in the dark
Stability under storage conditions	Stable
Expiry date	01 January 2003

The sponsor is responsible for all test substance data unless determined by NOTOX.

Note: Don't heat up the test substance above 50°C

PURPOSE AND PRINCIPLE

The purpose of the study was to evaluate the solubility of the test substance in water using the flask method.

GUIDELINES

The study procedure described in this report was based on the following guidelines:

Organization for Economic Co-operation and Development (OECD), OECD guideline for testing of chemicals no. 105: "Water Solubility", Adopted July 27, 1995.

European Economic Community (EEC), EEC-Directive 92/69 EEC, Part A, Methods for the determination of physico-chemical properties, A.6: "Water solubility". EEC Publication no. L383, December 1992.

ARCHIVING

NOTOX B.V. will archive the following data for at least 10 years: protocol, report, test substance reference sample and raw data.

TEST SYSTEM AND RATIONALE

Double distilled water.

The test system is recognised by the international guidelines (EEC, OECD).

VALIDATION OF THE TEST PROCEDURE

The test method as outlined in this report is validated periodically, using 4-nitrophenol and 2,4-dichloro phenoxy acetic acid alternately. The results were in accordance with the criteria of OECD Guideline No.105, "Water solubility" (1981).

REAGENTS

Acetonitrile	HPLC-grade, Labscan Limited Co., Dublin, Ireland
Milli-Q water	Tap water purified by reversed osmosis and subsequently passed over activated carbon and ion-exchange cartridges; Millipore Corp., Bedford, MA, USA

PERFORMANCE OF THE TEST

Preliminary tests

Two preliminary tests were carried out prior to performance of the flask method.

Preparation of test solutions:

In an Erlenmeyer flask, 20 ml double distilled water was added to 23.0 g test substance. In a second flask, 20 ml double distilled water was added to 12.1 g test substance. The flasks were placed on a magnetic stirring device in a climate room where the temperature ($20.0 \pm 0.5^{\circ}\text{C}$) was measured continuously using a validated computerised system (Rees Scientific, NJ, USA). The content of the flask was stirred for 17 days.

pH measurements:

The pH of the water mixture was measured after removal of undissolved test substance (see 'method of chemical analysis - sample pretreatment preliminary test').

Main study (flask method)

Preparation of test solutions:

Approximately 25 g test substance was weighed into each of three Erlenmeyer flasks. To each flask, 25 ml double distilled water was added. The flasks were securely closed and placed on a magnetic stirring device in a climate room where the temperature ($20.0 \pm 0.4^{\circ}\text{C}$) was measured continuously using the Rees system. The content of each flask was stirred for 24, 48 or 72 hours.

Preparation of a blank:

25 ml double distilled water was stirred for 72 hours in the climate room. The blank solution was pretreated and analysed in the same way as the test substance containing water mixtures.

pH measurements:

The pH of each of the water mixtures (except for the blank) was determined after removal of undissolved test substance (see 'method of chemical analysis-sample pretreatment main study').

METHOD OF CHEMICAL ANALYSIS

The concentration of [REDACTED] was determined by High Performance Liquid Chromatography. The conditions used are described below.

Sample pretreatment preliminary tests

A sample was taken from the water mixture and centrifuged three successive times for 5 minutes at 3500 g and 20°C. After the second as well as after the third centrifugation step, a subsample was taken from the supernatant and diluted by a factor of 2, 25 and 500 with 50/50 (v/v) acetonitrile/Milli-Q water. Each dilution was analysed.

Sample pretreatment main study

From each Erlenmeyer flask, duplicate samples were taken and centrifuged three successive times for 5 minutes at 3500 g and 20°C. After the third centrifugation step, a subsample was taken from the supernatant and diluted by a factor of 2, 25, 200 and 1000 with 50/50 (v/v) acetonitrile/Milli-Q water. Each dilution was analysed.

Analytical method

Column	Zorbax RX-C18, 250 x 4.6 mm; d _p =5 µm (Chrompack, Middelburg, the Netherlands)		
Mobile phase A	Acetonitrile		
Mobile phase B	Milli-Q water		
Gradient program	Time (min.)	%A	%B
	0	46	54
	5	46	54
	10	100	0
	13	100	0
	14	46	54
	19	46	54
Flow	2 ml/min		
Detection wavelength	220 nm and 260 nm ¹		
Injection volume	100 µl		

Calibration solutions

On each day of analysis, calibration solutions in 50/50 (v/v) acetonitrile/Milli-Q water were prepared from two standard solutions of Trigonox R-938 in acetonitrile.

¹ 260 nm for MIPK and 220 nm for all other components.

where

n = number of measurements

$$[(\text{highest} - \text{lowest})/\text{mean}] * 100\%$$

Quantification

Due to the fact that DMP, which was used for preparation of this formulation and which is a very well known compound, cannot be determined in the same chromatographic run as the other compounds due to a large difference in sensitivity (see also Notox Project 338805 "Development and validation of an analytical method for [REDACTED]"), analyses were not based on this compound. The water solubility of DMP is given in the literature as 4000 ± 60 mg/l at 25°C (Howard PH et al, Environ Toxicol Chem. 4:653-61 (1985)).

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Response:

R = Peak area component [units]

Calibration curve:

On each day of analysis, a calibration curve was calculated for each component. For this, the responses measured for each calibration solutions were correlated with the corresponding concentration. Linear regression analysis (least squares method) was used to calculate the calibration curve. Where necessary, a weighting factor (1/concentration) was used.

$$R = a * C + b$$

R = response calibration solution [units]

C = concentration of [] in calibration solution [mg/l]

a = slope [units*/mg]

b = intercept [units]

Calibration curves were constructed using 6 concentrations between 100 mg/l and 5000 mg/l. For each concentration, two responses were used. The coefficient of correlation was always > 0.99.

Samples

Concentration of [] in the water samples (based on one component):

$$C = \frac{(R-b) * d * 10^{-3}}{a} \text{ [g/l]}$$

R = response sample for the component [units]

d = dilution factor

a = slope of the calibration curve [units*/mg]

b = intercept of the calibration curve [units]

Water solubility

The water solubility of a test substance is specified by the saturation mass concentration of the test substance in water at a given temperature and is expressed in g/l.

RESULTS

Table 1 and 2 summarise the analytical results of the preliminary tests and the main study, respectively. Figures 1 and 2 show representative HPLC chromatograms.

Calculations were performed using not-rounded concentrations. Therefore, some differences might be observed when re-calculating the values as mentioned in the tables below. The reported concentrations analysed are the mean of duplicate analysis. The maximum deviation between the two sample responses was < 10%. Sample responses were in the calibrated range unless otherwise mentioned.

Preliminary tests

Test substance was stirred with double distilled water for 17 days at $20.0 \pm 0.5^\circ\text{C}$ at a test substance:water ratio of 1:1 (v:v) and also at 1:2 (v:v). The analytical results for the supernatants after two and three successive times of centrifugation (3500 g, 5 min. and 20°C) are summarised in the table below.

Table 1 Results of the preliminary test – water solubility of [REDACTED]

Component	Concentration [g/l] of [REDACTED] analysed in the water mixture with the nominal concentration of			
	605 g/l		1148 g/l	
	2 x centrifugation	3 x centrifugation	2 x centrifugation	3 x centrifugation
Hydrogen peroxide	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
pH	3.3	3.3	3.1	3.1

¹ Calculated by extrapolation of the calibration curve.

Based on these results it was decided to centrifuge the samples from the main study three times.

Main study (flask method)

Test substance was stirred with double distilled water (in triplicate) at a nominal concentration of 1 g/ml. After each stirring period, the water mixture was still a two-phase system (water and test substance).

Duplicate samples were taken from each water mixture after 24, 48 or 72 hours of stirring. The supernatants obtained after three successive centrifugation steps (3500 g, 5 min., 20°C) were analysed for [REDACTED]. The temperature of the climate room was $20.0 \pm 0.4^\circ\text{C}$ during the performance of the test. The analytical results for the samples are summarised in Table 2. No (significant) concentrations were measured in the blank water mixture for the components of concern.

Table 2 Results of the main study - water solubility of Trigonox R-938

Stirring time [hours]	Concentrations of [REDACTED] analysed for duplicate samples [g/l]					pH
	Hydrogen peroxide	MIPK	MIPKP-T4	MIPKP-T3 peak 1	MIPKP-T3 peak 2	
24	1132	158	198	2.48	1.70	3.6
	1148	159	201	2.57	1.76	3.6
48	1343	196	186	2.55	1.77	3.5
	1393	198	187	2.51	1.74	3.6
72	1510	230	172	2.45	1.69	3.5
	1573	226	172	2.41	1.66	3.5

Discussion

After each stirring period (17 days; preliminary test or 24, 48 or 72 hours; main study), there was still a two-phase system (water and test substance). Analytical measurements on each the components of concern in the water phase gave the following results:

Hydrogen peroxide appeared to be the component with the highest water solubility. The preliminary test showed that at a test substance:water ratio of 1:1 (v:v) the concentrations analysed were a factor of 2 higher than at a test substance:water ratio of 1:2 (v:v). At both concentration levels, the concentrations analysed were far above the nominal concentration, indicating that hydrogen peroxide might be formed due to reaction of the other compounds with water. The main study confirmed this assumption and showed that hydrogen peroxide concentrations increased with stirring time. After 24 hours of stirring, the concentrations analysed were already higher than the nominal concentration of 1 g/ml (i.e. a test substance:water ratio of 1:1 (v:v)). Based on Hydrogen peroxide, [REDACTED] was determined to be miscible with water in at least a 1:1 (v:v) ratio.

Both MIPKP-T4 and MIPK appeared to be good water soluble substances. Based on the results from the main study, it was concluded that MIPKP-T4 decomposes with time, whereas MIPK seems to be a decomposition product. Based on MIPK, the water solubility of [REDACTED] was determined to be ≥ 230 g/l, based on MIPKP-T4, the water solubility of [REDACTED] was estimated to be ≥ 201 g/l (maximum concentrations found in the aqueous samples).

MIPKP-T3 peak 1 and MIPKP-T3 peak 2 appeared to be the least water soluble components in [REDACTED]. For both components, a slight decrease in concentration was observed over the duration of the main study. Therefore, the solubility of the test substance based on MIPKP-T3 peak 1 and MIPKP-T3 peak 2 is reported as the average of the 24 hours samples. Based on MIPKP-T3 peak 1, the water solubility of [REDACTED] was determined to be 2.53 g/l. Based on MIPKP-T3 peak 2, the water solubility of [REDACTED] was determined to be 1.73 g/l.

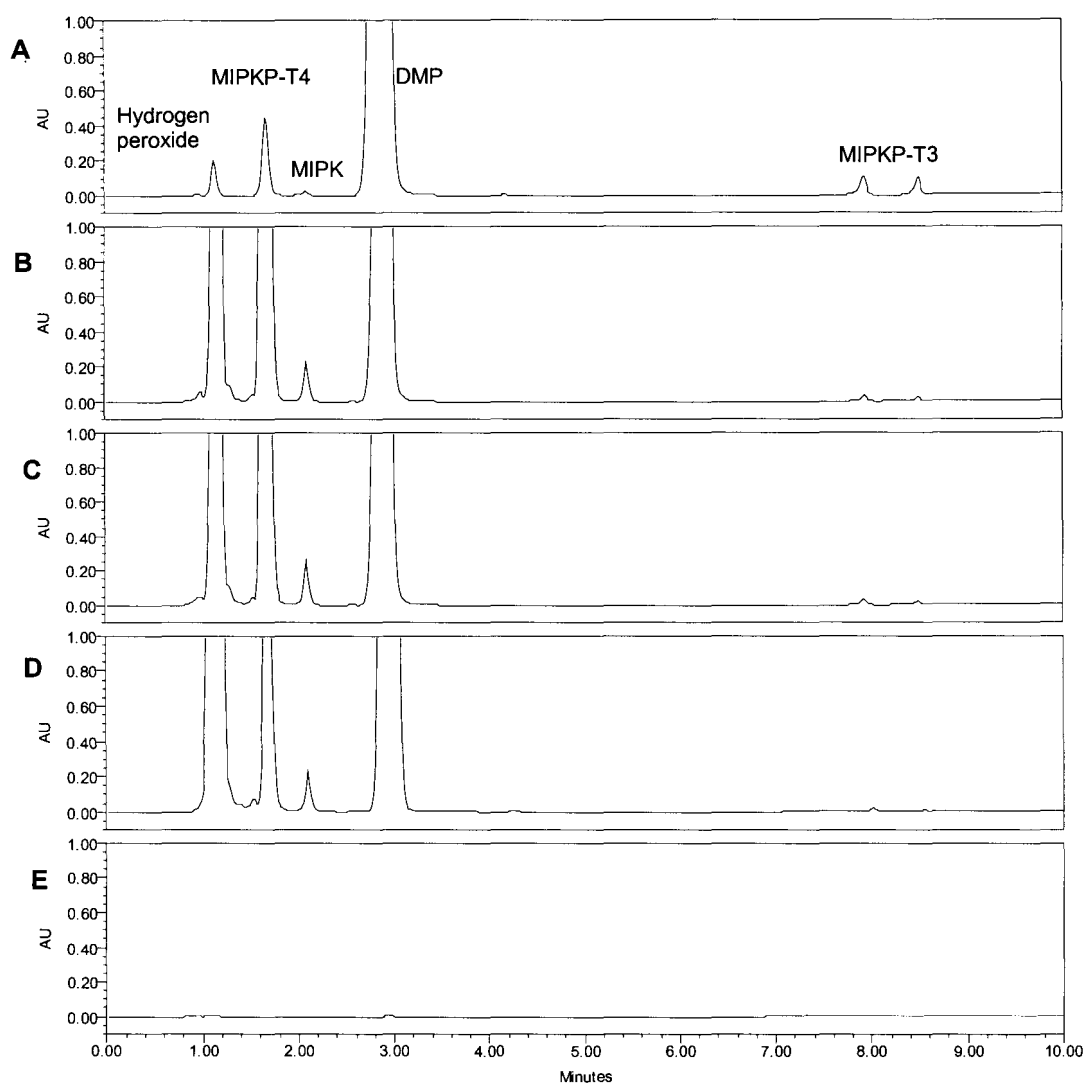


Figure 1 HPLC chromatograms of a calibration solution, of pretreated water samples after stirring periods of 24 hours (1 day), 72 hour (3 days) and after 17 days, and a chromatogram of a pretreated blank water sample (detection wavelength 220 nm).

- A:** The 4550 mg/l calibration solution [res.id. 2459]
B: Water sample after 24 hours (main study). Dilution factor: 2. [res.id. 2455].
C: Water sample after 72 hours (main study). Dilution factor: 2. [res.id. 2456].
D: Water sample after 17 days (prel. test). Dilution factor: 2. [res.id. 1582].
E: Blank water sample after 72 hours (main study). Dilution factor: 2. [res.id. 2422].

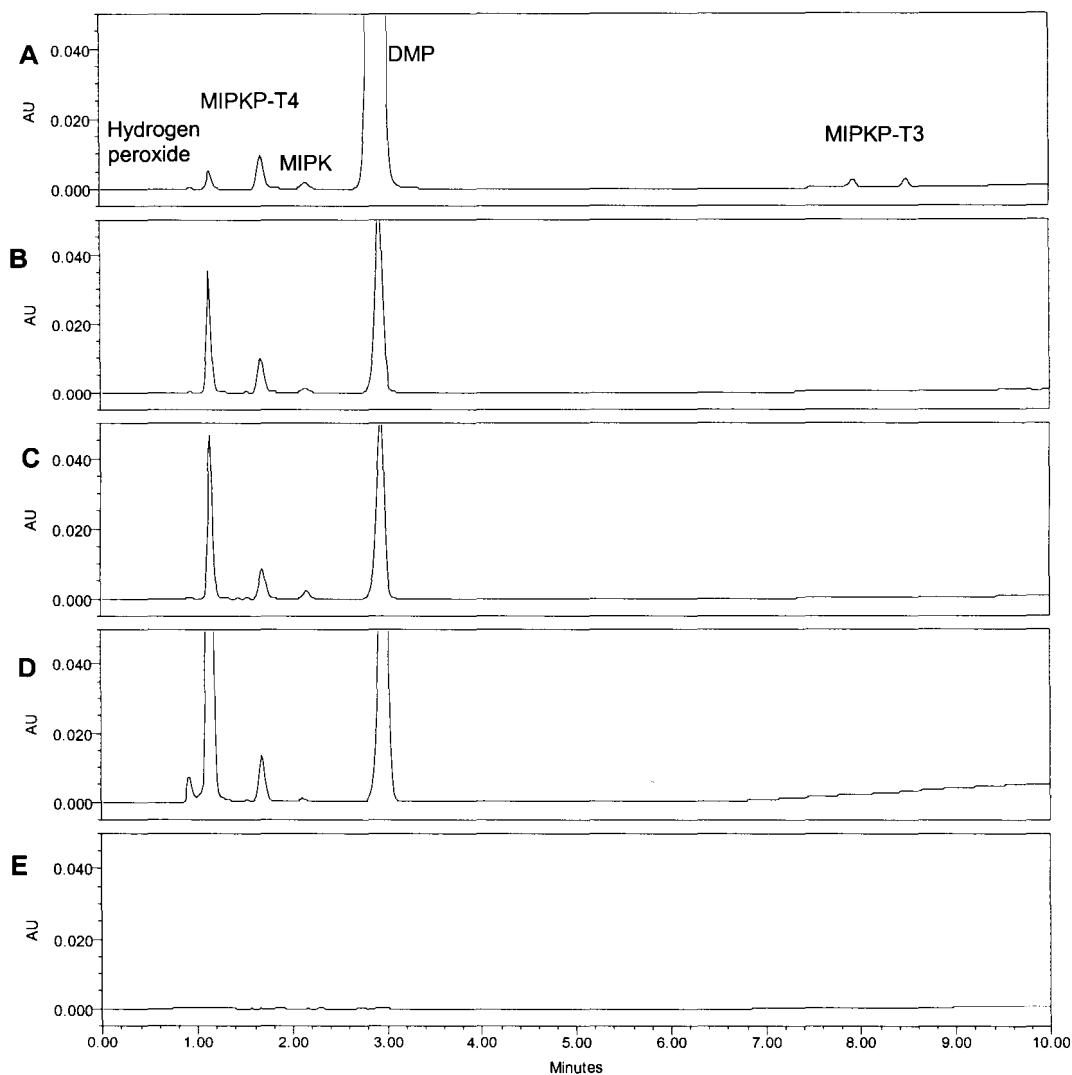


Figure 2 HPLC chromatograms of a calibration solution, of pretreated water samples after stirring periods of 24 hours (1 day), 72 hour (3 days) and after 17 days, and a chromatogram of a pretreated blank water sample (A,B,C and E: detection wavelength 260 nm; D: detection wavelength 220 nm)).

- A:** The 1001 mg/l calibration solution [res.id. 2263]
- B:** Water sample after 24 hours (main study). Dilution factor: 200. [res.id. 2283].
- C:** Water sample after 72 hours (main study). Dilution factor: 200. [res.id. 2291].
- D:** Water sample after 17 days (prel. test). Dilution factor: 500. [res.id. 1142].
- E:** Blank water sample after 72 hours (main study). Dilution factor: 200. [res.id. 2328].



CERTIFICATE OF ANALYSIS



Certificate of Analysis



page 1 of 2

ICS-331

Product name	:	
Chemical name	:	
Batch number	:	

Test results:

Method	Analysis of	Unit	Result *1
Jo/72.11, Jo/95.2	Peroxidic compounds (sum) <i>See page 2 for a specification</i>	% m/m	28.6 (± 1.5)
J20010792			

*1 bracketed values are estimated 95% confidence intervals

File code :
Analytical documentation : 20010792

Authorized by

[Redacted]

[Redacted]

Certificate of Analysis

[Redacted]

[Redacted]: specification of the peroxidic compounds

structure	% m/m
[Redacted]	
[Redacted]	
[Redacted]	

[Redacted]